REMARKS/ARGUMENTS

Claims 1-2 and 21 are pending. No claims have been allowed.

Claim 1 has been amended to claim a composition in which the antibodies specific for the 16 and 30 kDa antigens are in the "pharmaceutically acceptable carrier" of Claim 3 and "the antibodies are from serum from an animal immunized with the antigen". Support for pharmaceutically acceptable carriers can be found on page 14 of the specification and support for preparing polyclonal and monoclonal antibodies by immunizing animals with the antigen can be found on pages 26-27 and Example 1 of the specification.

Pages 26-27 discuss making polyclonal antibodies by injecting a suitable host (preferably, a horse, swine, rabbit, sheep, or goat) with the 16 and/or 30 kDa antigens (page 26, lines 27-32) to induce production of the antibodies specific for the 16 and/or 30 kDa antigens by methods well known in the art (page 26, line 32, to page 27, line 3). Methods for isolating antibodies such as ammonium sulfate precipitation, affinity chromatography, DEAE column chromatography are well known in the art.

Page 27, lines 4-22, and Example 1 discuss

making monoclonal antibodies against each of the antigens. Example 1 describes purifying the antigens by two-dimensional gel electrophoresis (page 33, lines 29-34), immunizing mice with the purified 16 and/or 30 kDa antigens (page 34, lines 7-10), and checking the mouse serum for antibodies specific for the 16 and/or 30 kDa antigens (page 34, lines 12-14). The serum contains polyclonal antibodies against the 16 and/or 30 kDa antigens which also supports the antibodies of Claim 1. The example then goes on to describe making monoclonal antibodies specific for the 16 and/or 30 kDa antigens from those mice which contain antibodies in their serum which is specific for the 16 and/or 30 kDa antigens.

The amendments to Claim 1 distinguish the composition from the serum and CSF of the prior art. The prior art serum and CSF contain antibodies induced by infection with *Sarcocystis neurona* whereas the applicants' currently claimed composition contains antibodies from serum from an animal in which the antibodies had been induced by immunizing the animal with the antigen, which can be purified from other *Sarcocystis neurona* antigens. Furthermore, the currently claimed composition includes a pharmaceutically acceptable carrier which is a component

of the serum or CSF of Liang.

Claim 2 has been amended to limit the composition of Claim 1 to antibodies which are monoclonal antibodies specific for the 16 and 30 kDa antigens.

Claim 21 has been amended to recite providing antibodies specific for the 16 and 30 kDa antigens in the "pharmaceutically acceptable carrier" of Claim 22 and "wherein the antibodies are from serum from an animal inoculated with the antigen". Accordingly, Claim 22 has been cancelled.

The amendments have narrowed the claims to compositions and are not believed to be substantive in nature. The amendments were made either to put the claims in form for allowance or to narrow the issues for appeal.

A Declaration Under 37 C.F.R. § 1.132 is enclosed which demonstrates that in contrast to the prior art of record (Liang), at least some antibodies against the 30 kDa antigen are neutralizing.

1. Claims 1-3 remain rejected under 35 U.S.C. § 102(b) as being anticipated by Liang.

Currently amended Claims 1 and 2 are not

believed to be anticipated by Liang under 35 U.S.C. § 102(b). The applicants' currently amended claims claim compositions which comprise a mixture of isolated antibodies against the 16 and 30 kDa antigens in a pharmaceutically acceptable carrier and wherein the antibodies are from serum from an animal inoculated with the antigen. Currently amended Claim 2 consists essentially of a mixture of monoclonal antibodies against the 16 and 30 kDa antigens. Claim 3 has been cancelled.

Liang shows in Figure 1 that horses with EPM have serum containing antibodies against various combinations of the 11, 14, 16, and 30 kDa antigens. Some sera and CSF contain only antibodies against 16 and 30 kDa antigens whereas other sera also contains antibodies against the 11 and 14 kD antigens as well. While Liang's serum and CSF are filtered to remove infectious contaminants, the serum and CSF still contains a variety of other serum constituents such as antibodies against a variety of other pathogens. In contrast, in the applicants' currently claimed composition the antibodies are from serum from an animal inoculated with the antigen and the composition includes a pharmaceutically acceptable carrier. Furthermore, in

Claim 2, the composition comprises monoclonal antibodies against the antigens. Serum and CSF from horses with EPM certainly do not contain monoclonal antibodies as set forth in Claim 2.

In light of the above, the applicants' currently claimed composition as set forth in Claim 1 or Claim 2 is distinguishable from the serum and CSF of Liang and thus, not anticipated by Liang. Reconsideration of the rejection is requested.

2. Claims 21 and 22 remain rejected under 35 U.S.C. § 112, first paragraph, as not being enabled by the specification.

The applicants believe that the specification provides enablement which is commensurate with the scope of currently amended Claim 21.

The currently amended claims provide a method for treating horses infected with *Sarcocystis neurona* by providing a mixture comprising antibodies against the 16 ±4 and 30 ±4 kDa antigens in a pharmaceutically acceptable carrier and inoculating the horse with the mixture. When all of the evidence relating to the factors set forth in M.P.E.P. § 2164.01(a) for determining whether a disclosure satisfies the

enablement requirement is considered, the evidence as a whole shows that the scope of the applicants' presently amended claims is enabled by the applicants' disclosure.

In addition to the arguments, made in the previous Amendment (Paper No. 7) the applicant adds the following.

As taught in Liang, most horses exposed to *Sarcocystis neurona* do not develop clinical EPM which suggests that the horses have developed effective immunity which may have prevented entry of the parasite into the central nervous system (CNS). Thus, it appears that horses with EPM have some defect in their immune response which allowed the parasite to enter the CNS.

Liang also teaches that the immunodominant antigens are the 11, 14, 16, and 30 kDa antigens. Liang further provides data which suggests that while antibodies against the 14 and 16 kDa antigens are neutralizing, antibodies against the 30 kDa antigen are not neutralizing.

In contrast to the data in Liang, the applicants have data which show that at least some antibodies against the 30 kDa antigen are neutralizing. The data are presented in an Declaration under 37 C.F.R. § 1.132 which clearly shows not only that some CSF

antibodies against the 30 kDa antigen are neutralizing but that CSF containing antibodies against both the 16 and the 30 kDa antigens appeared to be more neutralizing than either antibody species alone. The applicants' data shows that a composition containing antibodies against both the 16 and 30 kDa antigens would be reasonably expected to provide an effective treatment for horses infected with *Sarcocystis neurona*. The applicants' data shows that if one skilled in the art had relied upon the teachings of Liang for guidance, they would have mistakenly believed that antibodies against the 30 kDa antigen were non-neutralizing.

It is important to note that in Liang, serum and CSF samples were obtained from horses with a clinical diagnosis of a neurologic disorder resembling EPM. As taught by the applicants, horses with lameness or other neurologic diseases are being misdiagnosed as having EPM (page 4, lines 16-17). Since Liang does not demonstrate that the horses with clinical signs resembling EPM were actually infected with *Sarcocystis neurona* it is not known whether any of the Liang samples reported to contain only antibodies against a 30 kDa antigen (Liang: Figure 2) were infected with *Sarcocystis neurona*. The horses might have been

infected with another *Sarcocystis* species which induces an antibody that reacts non-specifically with the 30 kDa antigen from *Sarcocystis neurona*. For example, the applicants show in U.S. Patent No. 6,153,394 to Mansfield *et al.*, which had been incorporated by reference on page 13, lines 16-20, that the 16 and 30 kDa antigens are *Sarcocystis neurona*-specific (Mansfield: col. 7, lines 37-46; Figure 4), that serum from horses known not to be infected with *Sarcocystis neurona* contain antibodies which in Western blots appear to cross-react with the 16 and 30 kDa antigens (Mansfield: col. 6, lines 43-50; Figure 3), and that the observed antibody cross-reactivity might be because antibodies to other apicomplexian species can occur at or near the about 12 and 29 kDa bands and therefore may cause false-positive test results (Mansfield: col. 3, lines 11-19). Thus, Liang's support for the statement that antibodies against the 30 kDa antigen are not neutralizing is equivocal at best. Therefore, Liang is not believed to provide suitable support for the rejection.

Nevertheless, in light of the applicants' disclosure and declaration and other teachings in Liang, it would appear to be reasonable to believe that horses

with EPM have an inadequate immune response to the parasite which is not sufficient to prevent entry of the parasite into the CNS and that boosting the immune response with antibodies against the 16 and 30 kDa antigens might provide a sufficient boost to an infected horse's immune response to inhibit entry of the parasite into the CSF. The applicants' currently claimed method provides such a means for treating such infected horses. The applicants' composition would boost the concentration of antibodies against the 16 and 30 kDa antigens in the horse. It would be reasonable to expect that the increased level of antibodies against those two antigens would have a beneficial effect on the horse such as preventing further entry of the parasite into the CNS even if the increased level of antibodies did not cure the horse of the parasite.

Therefore, in light of the above, currently amended Claim 21 is believed to be enabled by the applicants' disclosure. Reconsideration of the rejection is requested.

3. In view of the above, it is believed that Claims 1, 2, and 21 are patentable. Notice of allowance is requested.

Respectfully,

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Encl. Declaration Under 37 C.F.R. § 1.132

APPENDIX B

MSU 4.1-526
Appl. No. 09/670,096
April 1, 2003
Reply to Office Action of Jan. 23, 2003

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Linda S. Mansfield, Mary G. Rossano, Alice J. Murphy, and Ruth A. Vrable

Serial No. 09/670,096 Group Art Unit: 1645

Filing Date: 2000 September 26

Title: VACCINE TO CONTROL EQUINE PROTOZOAL MYELOENCEPHALITIS IN HORSES

Examiner: Padmavathi Basker, Ph.D.

BOX AF

Commissioner of Patents and Trademarks

Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. § 1.132

Dear sir:

Alice J. Murphy states as follows.

(1) That she is an inventor of the invention in the above entitled application.

(2) That she performed an experiment in East Lansing, Michigan at Michigan State University (assignee of the present invention) to determine the neutralizing ability of antibodies against the 16 and 30 kDa antigens. The results showed that cerebral spinal fluid (CSF) from horses infected with *Sarcocystis neurona* which contained only antibodies that were strongly reacting against the 30 kDa antigen was neutralizing as was CSF which contained only antibodies that were strongly reacting against the 16 kDa antigen.

(3) That the experiment used CSF samples isolated from three horses infected with *Sarcocystis neurona*. CSF from the first infected horse contained antibodies which strongly

reacted against both the 16 kDa and 30 kDa antigens, CSF from the second infected horse contained antibodies which strongly reacted against only the 30 kDa antigen, and CFS from the third infected horse contained antibodies which strongly reacted against only the 16 kDa antigen. The controls for the experiment consisted of CSF from a horse from India known not to be infected with *Sarcocystis neurona* and Tris-buffered saline (TBS) containing 5% fetal bovine serum (FBS). The first horse was also culture positive for *Sarcocystis neurona*. Neural tissue from the horse at necropsy was ground up and inoculated into the media on equine dermal cells in culture. The media was replaced after 24, 48, and possibly 72 hours post inoculation and then weekly thereafter. The first plaque was seen on day 29 after inoculation. The merozoites from the plaques were subsequently identified as *Sarcocystis neurona* by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) techniques. Horses 2 and 3 had clinical signs which suggested the horses were infected with *Sarcocystis neurona*.

(4) That the experiment was performed as follows.

(a) Merozoites of *Sarcocystis neurona* from culture were washed in Tris-buffered saline (TBS) twice to remove media. The merozoites had been previously obtained from neural tissue from a Michigan horse infected with *Sarcocystis neurona*. The identity of the merozoites had been confirmed by PCR and RFLP.

(b) The washed merozoites were diluted in TBS and 35 μL was added to each of the 6 tubes comprising each of the horse groups. To test viability of the merozoites, 35 μL of the merozoites were affixed to a slide by cytospin (two replicates) and stained. There appeared to be about 20 to 30 viable merozoites per 35 μL. The stained cytospin provided an idea of the number of normal appearing and potentially viable merozoites per 35 μL aliquot. To confirm the viability of the merozoites, a real viability test was performed as follows. 70 μL of the merozoites were added directly to a 25 mL flask of confluent equine dermal cells. An additional 70 μL of merozoites were washed and spun twice in the same manner as the test samples. The pellet was

suspended in media and divided between two 25 mL flasks of confluent equine dermal cells. Two hours after inoculating the flasks, moving (spinning as they do when they are "drilling" into a cell) merozoites were seen in all three flasks. In addition, all three flasks developed plaques. Plaques consisted of a minimum of three rounded-up cells contiguous with one another to a maximum of a bare area of surface surrounded by rounded-up cells. (Cells round up when infected and come loose off the well or flask when the cell is heavily laden with parasite or the cell bursts from the parasite load. Since infective merozoites tend to move only into neighboring cells (unless one shakes up the flask which happens when the media is changed), bare areas surrounded by rounded up cells in older plaques are seen). The test for real viability confirmed that the merozoites used in the experiment described herein were viable.

(c) The CSF sample from horse 1 was diluted 1:10, 1:20, 1:40, 1:80, 1:160 with TBS and the ~~CFS~~ CSF samples from horses 2 and 3 were diluted 1:10, 1:20, 1:40, 1:80 with TBS. 200 μL of undiluted CSF and each dilution of CSF was each added to a tube of merozoites. The controls consisted of undiluted Indian horse CSF and TBS containing 5% FBS. There were six replicates of each of the samples and controls.

(d) All the samples and controls were incubated for one hour at 37° C.

(e) Each tube was centrifuged for 4 minutes at 1000 xg to pellet the merozoites. The supernatant fraction was removed and the merozoites were washed by resuspending the merozoite pellets in 300 μL TBS and centrifuging to pellet the merozoites and removing the supernatant fraction. Two washes were performed.

(f) After the final wash, the merozoite pellets were each resuspended in 200 μL of media and then each suspension was added to a well of a six–well plate of a monolayer of equine dermal cells which was just confluent.

(g) The plates were gently swirled to distribute the merozoites over the

monolayer and the cells then incubated at 37° C in a 5% CO_2 atmosphere. The media was replaced after 24 hours and then replaced weekly thereafter.

(h) Any plaques which formed were counted at five and six weeks post inoculation.

(5) That the results of the experiment are shown in Table 1; that the results show that the CSF containing antibodies against either antigen was separately neutralizing when used undiluted compared to the controls; that the results further show that CSF containing both antibodies was neutralizing even when used at a 1:10 dilution; and, that the results show that the neutralizing ability of the undiluted CSF from all three infected horses appears to be significant as was the 1:10 dilution of the CSF from the first horse.

Table 1

Sample	Dilution	Mean No. Plaques (SE) at 5 weeks post inoculation	Mean No. Plaques (SE) at 6 weeks post inoculation
1	undiluted	**4.3 (0.5)**	**20.0 (1.2)**
(Anti-16 & -30)	1:10	**3.2 (0.6)**	**30.0 (1.8)**
	1:20	6.0 (0.6)	55.5 (2.7)
	1:40	7.2 (0.4)	54.2 (2.8)
	1:80	9.0 (0.6)	52.7 (1.7)
	1:160	8.8 (0.4)	54.5 (2.3)
2	undiluted	**3.7 (0.4)**	**37.5 (2.4)**
(Anti-30)	1:10	7.8 (0.3)	57.5 (2.5)
	1:20	6.8 (0.3)	53.2 (3.0)
	1:40	8.3 (0.8)	53.2 (1.7)
	1:80	9.8 (0.4)	57.8 (3.1)
3	undiluted	**4.0 (0.6)**	**36.3 (2.1)**
(Anti-16)	1:10	7.7 (0.8)	55.3 (2.5)
	1:20	8.7 (0.6)	58.3 (3.1)
	1:40	8.7 (0.9)	55.5 (2.6)
	1:80	7.7 (0.8)	49.2 (2.4)
Indian horse	undiluted	8.7 (0.4)	56.8 (2.7)
5% FBS	undiluted	8.5 (0.6)	54.8 (3.2)

(6) That the results of Liang *et al.*, published in Infection and Immunity 66: 1834-1838 (1998), which shows that antibodies against the 30 kDa antigen in serum or CSF from horses infected with *Sarcocystis neurona* are not neutralizing, are not consistent with the results described herein and are not believed to be correct.

(7) That the undersigned declares further that all statements made herein of her own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issuing thereon.

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Appl. No. 09/670,096
April 1, 2003
Reply to Office Action of Jan. 23, 2003

Alice J. Murphy

Alice J. Murphy

Date: 4-17-03